Novel Preparation of a 2′-*O*-Acetyl-1′-*O*-(4-methoxybenzyl)-L-biopterin Derivative, a Versatile Precursor for a Selective Synthesis of L-Biopterin Glycosides

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Abstract: L-Rhamnose was converted, over a 13-step-sequence, into 2′-*O*-acetyl-N^2-(*N*,*N*-dimethylaminomethylene)-1′-*O*-(4-methoxybenzyl)-3-[2-(4-nitrophenyl)ethyl]-L-biopterin, an appropriately protected precursor of 1′-*O*- and 2′-*O*-monoglycosyl-L-biopterin. Thus, the first selective synthesis of these L-biopterin glycosides was accomplished by treatment of the precursor with either DDQ or sodium methoxide, then with tetra-*O*-benzoyl-α-D-glucopyranosyl bromide in the presence of silver triflate and tetramethylurea, followed by removal of the remaining protecting groups.

Key words: L-biopterin glycosides, limipterin, glycosylations, pteridine, protecting groups

Various pterin derivatives have been isolated from many living organisms, including microorganisms, algae, insects, fish, amphibians, and mammals.[1] Among the pterin derivatives, L-biopterin (**1**) is the most abundant of the naturally occurring pterins found in human urine[2] and exhibits enzyme cofactor activity in hydroxylation of aromatic amino acids as the form of its tetrahydro derivative.[3] Meanwhile, pterin glycosides having various kinds of sugars attached to the side-chain at C-6 of the pteridine ring were found to be produced by some prokaryotes. For example, 2′-*O*-(α-D-glucopyranosyl)-L-biopterin (**2**) was isolated from cyanobacterium, *Anacystis nidulans*,[4] *Synechococcus* sp. PCC 7942,[5] and *Spirulina (Arthrospira) platensis*,[6] whereas limipterin [2′-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-biopterin] (**3**) was isolated from a green sulfur photosynthetic bacterium *Chlorobium limicola f. thiosulfatophilum* NCIB 8327 (Figure 1).[7] Besides these glycosides of L-biopterin, some glycosides of other pterin derivatives have also been found in nature[8] and some of them have remained obscure as for the anomeric structure of the sugar moiety and the position of the pterin moiety where the sugar attaches.[9]

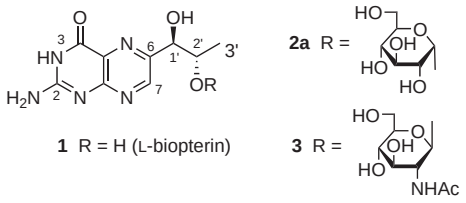
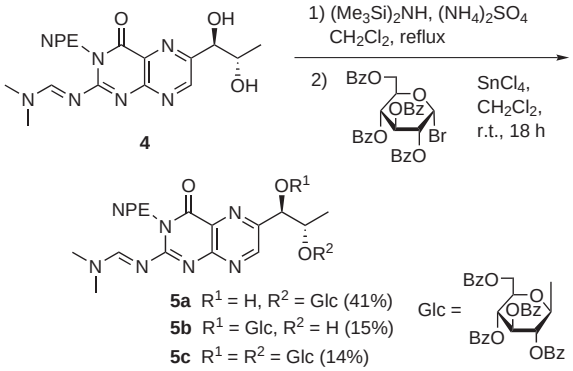


Figure 1

Efficient preparation of various types of glycosides of biopterin and related pterins by glycosylation has not been achieved so far, despite considerable interest from the viewpoint of their physiological function and biological activities as well as the structural proof of hitherto reported natural products. Although we reported the synthesis of 2′-*O*-(D-glucopyranosyl)-L-biopterins,[10] glycosylation of N^2-(*N*,*N*-dimethylaminomethylene)-3-[2-(4-nitrophenyl)ethyl]-L-biopterin (**4**) was not obtained with high selectivity: e.g., treatment of **4** with tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[11] (3 mol equiv) in the presence of tin(IV) chloride afforded 2′-*O*-(β-D-glucopyranosyl)-L-biopterin (**5a**, 41% yield), together with the 1′-*O*-glycosyl isomer **5b** (15%) and the 1′,2′-di-*O*-glycosyl derivative **5c** (14%; Scheme 1).



Scheme 1

This result prompted us to undertake an effective preparation of 1′-O- and 2′-O-monoprotected L-biopterin derivatives as the potential key precursors to achieve the selective 2′-O- and 1′-O-monoglycosylation. Although synthetic procedures for biopterin itself[12] and protection of the pyrimidine ring moiety[13] were well documented, preparation of biopterin derivatives whose one hydroxy group of the side-chain diols is protected has not been reported yet, to the best of our knowledge. Taking into consideration the available combination of protecting groups employed for the synthetic pathways, we have chosen *p*-methoxybenzyl (PMB) group for protection of 1′-hydroxy group, since its cleavage can be effectively performed under specific conditions[14] that cause no disruption of the rest of the molecule. We now describe

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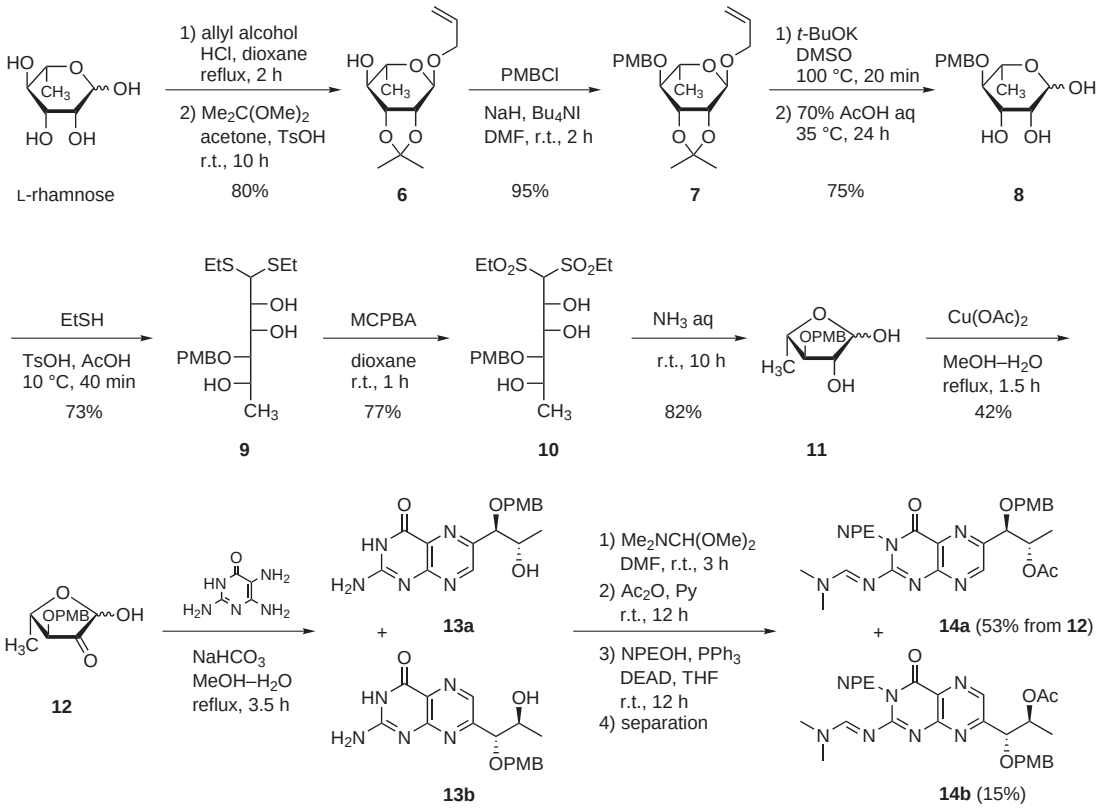
herein the preparation of a novel versatile 2′-*O*-acetyl-1′-*O*-PMB-L-biopterin derivative and the first selective glycosylation of L-biopterin.

Since no example of 3-O-protected 5-deoxy-L-arabinoses has been reported so far, we designed a synthetic route for the key intermediate 5-deoxy-3-*O*-PMB-L-arabinose (**11**) by starting from L-rhamnose (Scheme 2). Thus, glycosidation of L-rhamnose with allyl alcohol and the subsequent acetalation with 2,2-dimethoxypropane provided allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**6**,[15] 80%) together with the corresponding β-anomer (8%). Treatment of **6** with *p*-methoxybenzyl chloride and sodium hydride in DMF gave the 4-*O*-PMB derivative **7**. Conversion of the allyl glycoside **7** into the 1-propenyl glycoside with potassium *tert*-butoxide in DMSO, followed by hydrolysis in 70% acetic acid,[16] afforded 4-*O*-PMB-L-rhamnopyranose (**8**).

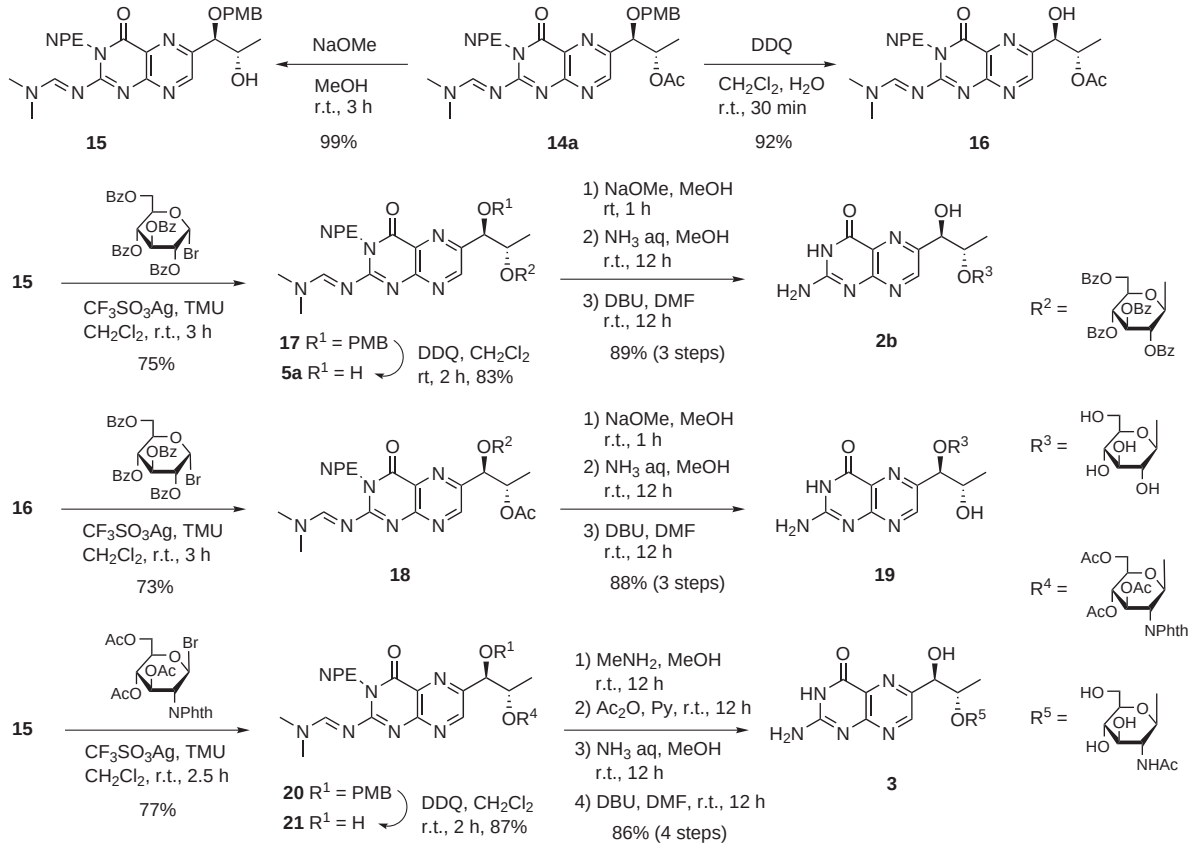
The cleavage of C-1 of **8** was achieved by application of the Hough and Taylor's procedures[17] with a slight modification. Namely, treatment of **8** with ethanethiol in the presence of tosylic acid in acetic acid gave the dithioacetal **9**, which was then oxidized with *m*-chloroperbenzoic acid (MCPBA) to the corresponding sulfone **10**. Degradation of **10** with dilute aqueous ammonia afforded 5-deoxy-3-*O*-PMB-L-arbinofuranose (**11**).

The selective oxidation for the 2-hydroxy group of **11** with cupric acetate[18] provided the L-*erythro*-pentos-2-ulose derivative **12**. The pteridine ring formation of **12** with 2,5,6-triamino-4-hydroxypyrimidine sulfate was carried out in aqueous sodium bicarbonate solution to give an inseparable mixture of the 6-substituted pterin **13a** and its 7-substituted isomer **13b**.[19] These products were separated and characterized after having been converted into the fully-protected derivatives **14a**,**b** by the following three steps. Namely, treatment of **13a**,**b** with *N*,*N*-dimethylformamide dimethyl acetal in DMF, the following acetylation of a hydroxy group afforded 2′-*O*-acetyl-N^2-(*N*,*N*-dimethylaminomethylene)-1′-*O*-PMB derivatives, whose N-3 position was then protected with 2-(4-nitrophenyl)ethyl (NPE) group by Mitsunobu reaction with NPE alcohol in the presence of triphenylphoshine and diethyl azodicarboxylate (DEAD) to provide **14a** and **14b**. These products were separated by column chromatography over silica gel into the desired 6-substituted pterin (L-biopterin) derivative **14a** (53% overall yield from **12**) and the 7-substituted (L-primapterin) derivative **14b** (15%).

The structural assignment of **14a** and **14b** was achieved primarily on the basis of their ^{13}C NMR spectral data.[20] The signals of C-6 and C-7 of 6-alkylkylpteridines generally appear at a similar field, whereas C-7 signals of 7-alkyl derivatives shifts to a lower field (ca. 20 ppm) from those of C-6.[21] Therefore, the close values of **14a** (C-6: δ = 150.71 ppm, C-7: δ = 149.88 ppm) and the distant values of **14b** (C-6: δ = 140.92 ppm, C-7: δ = 159.98 ppm) indicate the 6-substituted pterin for the former and the 7-substituted pterin for the latter.



Scheme 2



Scheme 3

Deprotection of 2′-*O*-acetyl-1′-*O*-PMB-L-biopterin derivative **14a** was then carried out under mutually exclusive conditions to give the mono-O-protected compounds (Scheme 3). Namely, methanolysis of **14a** in the presence of sodium methoxide provided the 1′-*O*-PMB derivative **15**, while treatment of **14a** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) afforded the 2′-*O*-acetyl compound **16**. The partially protected pterins **15** and **16** are important precursors for the 2′- and 1′-*O*-monoglycosyl derivatives, respectively. In addition, these compounds have such an advantage as to be sufficiently soluble in dichloromethane, although pterin derivatives having the side chain diols are sparingly soluble in nonpolar aprotic solvents.

Efficient glycosylation of **15** was exemplified by the condensation with tetra-*O*-benzoyl-α-D-glucopyranosyl bromide in the presence of silver triflate[22] and tetramethylurea (TMU) in dichloromethane at room temperature for three hours, affording 2′-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-biopterin derivative (**17**) as a sole product in 75% yield. The similar treatment of **16** afforded 1′-*O*-glycosyl analogue **18** in 73% yield. Moreover, glycosylation of **15** with 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimindo-β-D-glucopyranosyl bromide[23] provided 2′-*O*-(1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimindo-β-D-glucopyranosyl)-L-biopterin derivative **20** in 77% yield.[24] Cleavage of PMB group of **17** and **20** was performed by use of DDQ without harming glycosyl linkage, affording **5a** and **21**, respectively.

Removal of the protecting groups of **5a** and **18** was carried out by the successive treatment with sodium methoxide (to cleave all acyl groups), aqueous ammonia (to cleave the *N*,*N*-dimethylaminomethylene group), and then DBU (to cleave the NPE group) to give 2′-*O*-(β-D-glucopyranosyl)-L-biopterin (**2b**) and its 1′-*O*-glycosyl analogue **19** in ca. 90% (overall yield from **5a** and **18**), respectively. Similarly, removal of the phthaloyl group and the *N*,*N*-dimethylaminomethylene group of **21** with methylamine, followed by the action of acetic anhydride, afforded the fully-acetylated derivative, which was then treated with aqueous ammonia and then with DBU to give limipterin (**3**) in 86% (overall yield from **21**).

In summary, we have developed a novel effective way for selective preparation of L-biopterin glycosides via 2′-*O*-acetyl-1′-*O*-PMB-L-biopterin derivative **14a**. The 1′-*O*-PMB derivatives **15** and the 2′-*O*-acetyl compounds **16** derived from **14a** are regarded as highly useful precursors respectively for the 2′- and 1′-*O*-glycosyl-L-biopterin derivatives having various types of sugar moiety.

Acknowledgment

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(20) Selected NMR data for **14a**: ^{1}H (600 MHz, $CDCl_3$): δ = 1.23 (3 H, d, $J_{2',3'}$ = 6.6 Hz, H-3′), 4.77 (1 H, d, $J_{1',2'}$ = 4.4 Hz, H-1′), 5.36 (1 H, qd, H-2′), 8.96 (1 H, s, H-7). ^{13}C (151 MHz, $CDCl_3$): δ = 15.92 (C-3′), 71.76 (C-2′), 82.21 (C-1′), 128.36 (C-4a), 149.88 (C-7), 150.71 (C-6), 153.63 (C-8a), 157.55 (C-2), 161.83 (C-4).
Selected NMR data for **14b**: ^{1}H (600 MHz, $CDCl_3$): δ = 1.22 (3 H, d, $J_{2',3'}$ = 6.6 Hz, H-3′), 4.66 (1 H, d, $J_{1',2'}$ = 4.2 Hz, H-1′), 5.37 (1 H, qd, H-2′), 8.78 (1 H, s, H-6). ^{13}C (151 MHz, $CDCl_3$): δ = 15.61 (C-3′), 71.94 (C-2′), 82.26 (C-1′), 129.29 (C-4a), 159.98 (C-7), 140.92 (C-6), 153.17 (C-8a), 157.88 (C-2), 161.83 (C-4).

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(24) **General Procedure for Glycosylation of 15.**
To a solution of **15** (56 mg, 0.10 mmol), glycosyl bromide (0.30 mmol) and TMU (0.012 mL, 0.10 mmol) in dry CH_2Cl_2 (1.0 mL) was added silver triflate (56 mg, 0.22 mmol). The mixture was stirred at r.t. for 3 h, diluted with $CHCl_3$, and filtered through Celite®. The filtrate was washed with aq $NaHCO_3$, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by column chromatography to give the 2′-*O*-glucopyranosyl-L-biopterin derivative.